

September 2011

Cost £7.21



Project Report No. 479

Breeding oilseed rape with a low requirement for nitrogen fertiliser

by

Pete Berry¹, John Foulkes², Pedro Carvalho², Graham Teakle³, Philip White⁴, Charlotte White⁵
and Susie Roques⁶

¹ADAS High Mowthorpe, Duggleby, Malton, North Yorkshire, YO17 8BP

²University of Nottingham, Division of Environmental and Agricultural Sciences, Sutton Bonington Campus, nr Loughborough, Leicestershire, LE12 5RD

³University of Warwick, Warwick Crop Centre, School of Life Sciences, Wellesbourne, CV35 9EF

⁴The James Hutton Institute, Craigiebuckler, Aberdeen, AB158QH

⁵ADAS Gleadthorpe, Meden Vale, Mansfield, Nottinghamshire, NG20 9PD

⁶ADAS Boxworth, Battlegate Rd, Boxworth, Cambridge, CB23 4NN

This is the final report of a 62 month project (RD-2004-3116) which started in July 2006. The work was funded by Defra and industry through the Sustainable Arable LINK Programme. The industry consortium included Northeast Biofuels Ltd, Limagrain UK Ltd, Elsoms Seeds Ltd, Saaten Union UK Ltd, Syngenta Seeds Ltd, BASF plc, GrowHow UK Ltd, BP Oil International Ltd and HGCA. The total project costs were £968,830 with in-kind contributions of £9,000 from HGCA.

While the Agriculture and Horticulture Development Board, operating through its HGCA division, seeks to ensure that the information contained within this document is accurate at the time of printing no warranty is given in respect thereof and, to the maximum extent permitted by law, the Agriculture and Horticulture Development Board accepts no liability for loss, damage or injury howsoever caused (including that caused by negligence) or suffered directly or indirectly in relation to information and opinions contained in or omitted from this document.

Reference herein to trade names and proprietary products without stating that they are protected does not imply that they may be regarded as unprotected and thus free for general use. No endorsement of named products is intended, nor is any criticism implied of other alternative, but unnamed, products.

HGCA is the cereals and oilseeds division of the Agriculture and Horticulture Development Board.



CONTENTS

1.	ABSTRACT	4
2.	SUMMARY	5
2.1.	Introduction/Background and aims	5
2.1.1.	Project aim	5
2.1.2.	Objectives	5
2.2.	Methods	6
2.2.1.	2006/7 and 2007/8 experiments.....	6
2.2.2.	2008/9 and 2009/10 experiments.....	6
2.2.3.	Methods of genetic analysis.....	7
2.3.	Results	7
2.3.1.	Varieties with low N requirement.....	7
2.3.2.	Traits associated with yield under Low N supply	8
2.3.3.	Model of yield formation under limited N supply	9
2.3.4.	Rapid screening methods	10
2.3.5.	Genetic analysis.....	11
2.3.6.	Impact of low N varieties on gross margin and the environment	12
2.3.7.	Prospects for developing new varieties with low N requirement	13
2.4.	Discussion	14
2.4.1.	Conclusions.....	14
2.4.2.	Future R&D resulting from this project	15
2.4.3.	Industrial relevance and plans for future commercial exploitation	16
2.4.4.	Publications.....	17

1. ABSTRACT

The relatively small number of previous studies into oilseed rape nitrogen use efficiency, together with the increasing cost of nitrogen fertiliser and concern about greenhouse gas emissions and nitrate leaching, create a need for an improved understanding of the varietal traits associated with yield under low nitrogen conditions in oilseed rape. This project aimed to address this need using a series of field experiments to understand the physiological mechanisms and identify the traits which determine yield differences between varieties grown under low nitrogen supply. A further objective involved developing methods which plant breeders could use to rapidly select varieties with low nitrogen requirement.

Statistically significant interactions were found for seed yield between variety and the supply of N, indicating that different traits are required to maximise yield under Low N supply compared with High N supply. Differences between varietal yields under Low and High N supply were generally consistent between seasons indicating that the traits conferring high yield under Low N are heritable.

Traits for high yield under low N supply identified in field experiments included post-flowering N uptake, more seeds/m², high remobilisation of stem N to pods and seed, tall stature, late maturing, high seed moisture, high seed oil, low seed N concentration, high seed glucosinolate concentration. Post-flowering N uptake was one of the most important traits and this appeared to be determined by demand from the sink (seeds/m²). Economic optimum N rates varied by over 100 kg N/ha between elite varieties.

A genetic map was created for a relevant doubled haploid (DH) population from a cross between elite winter OSR varieties Rocket and Capitol which can be used to associate genetic markers with traits. Contrasting yield performance under low and high N supply was identified for specific Rocket x Capitol DH lines and Tapidor x Victor substitution lines which will help identify the genetic regions that control N requirement.

N efficient varieties require low N optima AND high yield in order to be economically competitive and to minimise GHG emissions. GHG emissions varied by 30% between elite varieties as a result of differences in N requirement and yield. Breeding varieties with a low N requirement will reduce the risk of nitrate leaching during the winter following oilseed rape harvest. It was estimated that the fertiliser N requirement of typical current varieties could be reduced by 58% without reducing yield potential, which would reduce GHG emissions by 39%.

2. SUMMARY

2.1. Introduction/Background and aims

UK winter oilseed rape (OSR) has a high nitrogen (N) fertiliser requirement relative to its yield. It has been estimated to have an on-farm nitrogen use efficiency (NUE; harvestable dry matter yield per unit available N) of 10 kg seed DM/kg N, compared with 21 to 27 kg DM/kg N for wheat and barley. N fertiliser is not only a major cost for crop production, but also the main source of greenhouse gas (GHG) emissions from arable farming. It has been estimated that N fertiliser use accounts for 79% of the GHG emissions from oilseed rape production, through a combination of energy used in manufacturing and nitrous oxide (N₂O) emissions after application.

The relatively small number of previous studies into OSR NUE, together with the increasing cost of N fertiliser and concern about GHG emissions and nitrate leaching, create a need for an improved understanding of the varietal traits associated with yield under Low N conditions in OSR. This project aimed to address this need using a series of field experiments to understand the physiological mechanisms and identify the traits which determine yield differences between varieties grown under low N supply. A further objective involved developing methods which plant breeders could use to rapidly select varieties with low N requirement.

2.1.1. Project aim

Enable plant breeders to select oilseed rape varieties with a low requirement for fertiliser N and quantify the extent to which genetic selection will reduce pollution and costs associated with growing oilseed rape.

2.1.2. Objectives

- SO1 Develop a mechanistic model of N requirement in oilseed rape
- SO2 Identify traits associated with Low N requirement in UK oilseed rape varieties
- SO3 Develop phenotypic screens and genetic markers for rapidly assessing the traits
- SO4 Quantify the impact of different varieties on pollution, energy balance and growing costs

2.2. Methods

2.2.1. 2006/7 and 2007/8 experiments

Experiments were set up at ADAS High Mowthorpe (HM), ADAS Rosemaund (RM), Elsoms Seeds (ES), Limagrain UK Ltd (LN), Saaten Union UK Ltd (SU), NK Syngenta Seeds Ltd (SS). Variety screening experiments were set up at the four Plant Breeding sites in both seasons, which included 29 varieties and 2 levels of fertiliser N. Detailed physiology experiments were set up at the two ADAS sites in both seasons, which included 10 varieties and 2 levels of fertiliser N. The N treatments included Low N for which either zero or 80 kg N/ha was applied and High N which received the amount recommended by RB209 for a commercial crop based on measurements of soil mineral N and crop N in late winter. Fertiliser N rates ranged from 90 to 250 kg N/ha between the sites. A N response trial was also carried out at HM which included 5 varieties and 6 N rates (0 to 350 kg N/ha). Measurements at all sites included seed yield, oil content, protein content, glucosinolates, plant height at flowering, date of flowering score, date of maturity score, early lodging score, late lodging (stem stiffness) score. At the ADAS sites more detailed measurements of growth and N uptake were carried out throughout the season.

2.2.2. 2008/9 and 2009/10 experiments

Experiments including 88 DH (doubled haploid) lines from a Rocket x Capitol (RxC) cross were set up at HM, ES and LN in 2008/9 and at RM, SU and SS in 2009/10. Experiments including 75 Tapidor-Victor substitution lines (TVSL) were set up at RM, SU and SS in 2008/9 and at HM, ES and LN in 2009/10. Trials at the ADAS sites were grown at both High N and Low N rates, while trials at the breeders' sites were at either High N or Low N only. The Low N treatment received N fertiliser at rates of either zero or 50 kg N/ha. The High N treatment received N fertiliser rates ranging from 168 to 270 kg N/ha. An N response trial was also carried out at HM which included 4 varieties and 6 N rates (0 to 350 kg N/ha). Measurements at all sites included seed yield, oil content, protein content, glucosinolates, plant height at flowering, date of flowering score, date of maturity score, early lodging score, late lodging (stem stiffness) score.

At the University of Nottingham glasshouse experiments were set up in both years to investigate four varieties (Temple, Borneo, Grizzly, Lioness) that had been shown in years 1 and 2 to contrast in terms of their response to N; Temple had a high yield at Low and High N supply, Lioness had a high yield at High N and relatively low yield at Low N, Borneo had a high yield at Low N and a relatively low yield at High N, Grizzly had a low yield at Low N and a relatively high yield at High N. High N and Low N treatments were investigated together with droughted (50% of optimum water supply post-anthesis) and fully watered treatments both at High N. The plants were grown in long tubes (100cm long x 15cm in diameter) with 4 replicates of each treatment. Measurements

included detailed assessments of crop growth and N uptake from the start of stem extension onwards together with root length density at crop maturity.

2.2.3. Methods of genetic analysis

Genetic map construction for the Rocket x Capitol doubled haploid (RCDH) population was performed using JoinMap version 4 (Kyazma, Wageningen, Netherlands). A total of 360 markers were screened (188 SSRs and 172 SRAPs). QTL analysis was performed using MapQTL version 6 (Kyazma, Wageningen, Netherlands). A total of 88 RCDH lines were analysed.

The location of introgressions of the Victor genotype in the background of Tapidor in the Tapidor x Victor substitution line (TVSL) population was carried out. This was achieved by screening the parent lines with 250 SSR markers and then screening the polymorphic markers against the TVSL population. The linkage group location of the Victor substitutions in each line was achieved by comparing allele sizes with mapped loci from other maps. A total of 75 TVSL lines were analysed.

2.3. Results

2.3.1. Varieties with low N requirement

The experiments showed that there were statistically significant interactions for seed yield between variety and the supply of N, i.e. some varieties performed relatively better under Low N supply and other varieties performed relatively better under High N supply. This indicates that different traits are required to maximise yield under Low N supply compared with High N supply. The differences between varietal yields under Low and High N supply were generally consistent between seasons which indicates that the traits conferring high yield under Low N and the traits for high yield under High N supply are heritable. Varieties which achieved a relatively high yield under Low N supply included; Borneo, ES Betty, FD502, Sun and Temple.

Two full N response experiments were carried out with N rates ranging from zero to 350 kg N/ha. These experiments allowed the economic optimum N rate to be calculated for each variety. In 2007/08 the economic optimum N rate varied by 30 kg N/ha between 5 varieties and in 2009/10 the optimum N rate varied by more than 100 kg N/ha between four different varieties. In each experiment, strong negative relationships were found between yield at nil fertiliser and the economic optimum N rate for each variety (Figure 1). This indicates that yield at Low N could be a useful indicator of N requirement. However, it does not indicate the yield potential at High N supply.

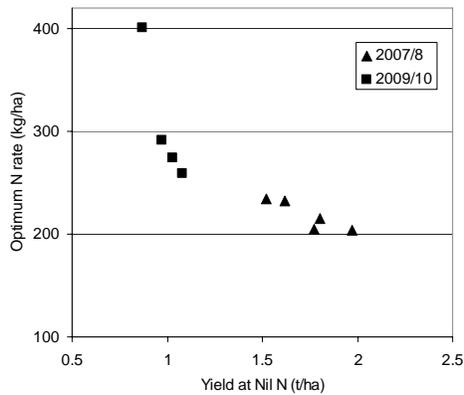


Figure 1. Relationship between yield at nil N and the optimum N rate for 5 varieties in 2007/8 and 4 different varieties in 2009/10.

2.3.2. Traits associated with yield under Low N supply

Traits associated with yield under Low N supply were investigated within the ADAS and Breeder experiments carried out in 2006/7 and 2007/8 and the Nottingham University experiments carried out in 2008/9 and 2009/10. Given that the N response experiments indicated that high yield under Low N supply was related to a smaller fertiliser N requirement for a variety then it is likely that the traits for high yield under Low N will be the same as those for low fertiliser N requirement.

The ADAS experiments demonstrated that varietal differences in yield under Low N were highly correlated with total N uptake by the crop, with each additional kg of N/ha taken up associated with an additional 20 kg of dry seed/ha. The majority of the variation in yield was explained by the amount of N taken up between mid-flowering and crop maturity. Each additional kg of N taken up after flowering resulted in an additional 16 kg of dry seed/ha. There was also a strong positive correlation between seeds/m² and yield at Low N. Each additional 1000 seeds/m² was associated with approximately 45 kg of seed dry matter/ha and with an additional 1.4 kg of post-flowering N uptake. It is possible that the relationship between seeds/m² and post-flowering N uptake was driven by the increase in seeds/m². Further evidence for this came from the 2008/9 experiments at Nottingham University in which incomplete pollination resulted in inter-plant variation in seeds per plant which was unrelated to varietal differences. It was shown that plants with more seeds also had more N uptake after flowering as well as greater re-mobilisation of N from the stem during seed filling.

Other traits which were positively related to high yield at Low N supply in the ADAS experiments included plant height, stem dry weight at flowering and total crop dry weight at mid-flowering. It is hypothesised that greater growth rate at around flowering may allow more seeds to be set which then stimulates greater post-flowering N uptake through a positive feedback mechanism. Greater late N uptake may then prolong the duration of the green canopy and the seed filling period.

Further research is required to understand the mechanism by which some varieties are able to set more seeds and prolong N uptake together with what traits underlie these traits.

Traits associated with high yield under Low N supply from the breeding experiments were generally consistent with the traits identified within the ADAS experiments with strong positive correlations for crop height, later maturity, greater seed moisture content, greater oil content and higher glucosinolate levels. There was also a negative correlation with seed protein content in several experiments. Traits which were either weakly or not correlated with yield at Low N in the ADAS, Breeder and Nottingham University experiments included seed size (modest correlations), N utilisation efficiency (kg of seed per kg of N taken up by the plant), N uptake at the start of stem extension and root length density.

2.3.3. Model of yield formation under limited N supply

A mechanistic model describing the formation of yield under sub-optimal N supply was developed using published information and new data from this current project. Yield production under limited N supplies is primarily dependent on the amount of N that the crop can take up. N uptake up to flowering is driven by the supply of N from the soil and fertiliser. N uptake post-flowering is driven by demand, which in turn, is determined by the number of seeds/m². The model is therefore co-driven by the supply of and demand for N.

The amount of N taken up by flowering is partitioned between the stem and leaf depending on variety factors. The proportion of this N which can then be remobilised to the developing pods is also dependent on variety factors. The amount of N taken up after flowering is dependent on the number of seeds/m² which, in turn, is positively related to variety differences in stem biomass and crop height at flowering as well as inherent variety differences in seeds/m². The amount of N available for pod development is used to estimate the GAI of the canopy during seed filling which, together with assumptions about radiation use efficiency, determines the rate of seed dry matter growth. Varietal differences in the seed N concentration then determine how quickly N must be remobilised from the pod walls and stems to the seed, with the duration of seed filling limited by either the amount of N available for seed growth or a specified maximum thermal duration for seed growth.

The N model was shown to produce realistic yield estimates at a range of N fertiliser levels. The N model was used to carry out a sensitivity analysis for the model inputs which have been shown in the LK0979 experiments to have reasonably consistent varietal differences across the trials. The varietal range for each trait has been taken from the LK0979 experiments. This analysis showed that under limited N conditions (zero N fertiliser) the following traits have the greatest influence on yield; post-anthesis N uptake, the proportion of stem N that could be relocated, seeds/m² and seed

N concentration. The relative importance of the traits for yield under low N conditions were generally consistent with the findings from the field experiments. A number of different traits were predicted by the model to be important in an environment where 200 kg N/ha fertiliser was applied including the duration of seed filling and radiation use efficiency of the pod canopy. Post-anthesis N uptake, seeds/m² and efficiency of soil N uptake were predicted to be slightly less important. In general there was a trend for N uptake related traits to be less important and N use efficiency traits to be more important under high N supply. It was estimated that if the best varietal traits observed for post-anthesis N uptake, seeds/m², seed N concentration, stem N remobilisation and a 10% increase in fertiliser uptake efficiency could be combined into one variety then the fertiliser requirement for high yields would be reduced by 40%.

2.3.4. Rapid screening methods

This project investigated simple visual screens, remote sensors and the potential to use genetic markers (see genetics section) as rapid screening methods. It was shown that a reasonable prediction of yield performance under Low N supply could be made from measurements taken on a standard variety trial grown at a commercial rate of N fertiliser by taking account of yield at High N supply, maturity score and seed glucosinolate content. Other easy-to-score traits which have been associated with greater yield under Low N supply include greater height, greater seed moisture, greater seed oil content and, less strongly, a lower seed protein concentration.

Remote sensing instruments that were investigated included a 'CropScan' multispectral radiometer which measured the light reflected back from the crop at 460 to 970 nm. Canopy temperature measurements were taken using an infra-red thermometer. Specific spectral reflectance indices or ratios which included the quantity of light reflected in the near-infra-red and visible spectrums gave the most reliable prediction of yields. One of the spectral reflectance indices gave a reasonable or good prediction of yield at Low N ($r > 0.5$) in 15 out of 20 experiments. It was shown that measurements should not be done in low levels of irradiance as this caused readings to vary systematically with time of measurement. Good yield prediction could be achieved from late flowering (the earliest growth stage assessed) until late seed filling. Measurements of canopy temperature gave good yield predictions at Low N in 7 out of 16 tests. It was shown that good predictions required near cloudless conditions as cloud cover caused the canopy temperature to fluctuate significantly. Several of the tests were affected by the presence of cloud. Successful yield predictions were achieved between late flowering and mid seed filling.

2.3.5. Genetic analysis

Two genetic populations were investigated including 88 DH (doubled haploid) lines from a Rocket x Capitol (RxC) and 75 Tapidor-Victor substitution lines (TVSL). These were grown at several sites in 2008/9 and 2009/10 using Low N and High N treatments. At High N, the parental line Capitol had a higher mean yield rank, 12, than Rocket which had a mean yield rank of 66. While at Low N, the mean yield ranks were more similar. Several lines showed a wide contrast in yield rankings between the Low N and High N treatments, e.g. across all experiments line RC25-054 had a mean rank of 4 at Low N and a mean yield rank of 52 at High N. For the TVSL population, the parental line Tapidor ranked 19th at Low N, whereas the other parental line Victor had a mean yield rank of 60 at Low N. At the High N treatment both parental lines had low yield ranks. TVSL58 showed the largest yield differences, having a mean yield rank of 5 at Low N and 68 at High N.

A genetic map was developed for the Rocket x Capitol DH population that comprised 22 linkage groups that corresponded to the 19 *B. napus* linkage groups. The map comprises 273 markers organised into 22 linkage groups that represent all 19 *B. napus* chromosomes. The linkage groups A01 and A05 are represented by 2 and 3 sub-linkage groups respectively as there was insufficient markers to connect them. The total map length is 1369 cM which corresponds to approximately 80% coverage compared with the Tapidor x Ningyou DH linkage map. Three significant yield QTL were identified together with a further five putative yield QTL. There was little consistency for the QTL between trials indicating strong genotype x environment interactions. QTL were also identified for several other traits including the oil, protein and glucosinolate concentrations in the seed, height, flowering date, early vigour and spectral reflectance indices. The genetic map generated was of sufficient quality to provide information on the genetic control of a number of the traits scored on the population and the density of markers on the map exceeded the original target of the project. It should be recognised however that more QTL could be identified with greater confidence if more DH lines were analysed and a more complete genetic marker map could be developed.

Work within OREGIN screened the TVSL population with 250 SSR markers to provide a skeleton outline of the position of the substitutions. This work has enabled the linkage group possessing a substitution to be tentatively assigned for the majority of lines. Lines have been identified that could be further analysed through additional crossing to reduce the size of the substitution interval in order to fine map the trait. The work to map the substitutions using SSRs in the OREGIN project has proved more difficult than anticipated due to a low SSR polymorphism rate and the observation that the Victor parent was not homozygous. However, with the recent advances in genotyping and sequencing technologies, it is anticipated that the substitutions will be located with high precision in the near future which will enable a more thorough evaluation of the data from this project.

2.3.6. Impact of low N varieties on gross margin and the environment

The large varietal differences in the economic optimum N rate between elite varieties resulted in large differences in gross margin of up to £369/ha. In both N response experiments the gross margins were closely and positively correlated with the yield at the economic N rate. However, there were some exceptions, e.g. in 2009/10 Lioness had the greatest yield of 4.96 t/ha, but its high N optima of 307 kg N/ha caused it to have a £52/ha lower gross margin than Temple which yielded 4.91 t/ha and had an N optima of 245 kg N/ha. Nonetheless, Lioness still had the 2nd highest gross margin. These results demonstrate that for low N varieties to be economically viable then there is little or no tolerance for a trade-off in yield potential, and breeders must concentrate on producing varieties with a low N requirement AND high yield.

The large varietal differences in the economic optimum N rate between elite varieties also resulted in large differences in N use efficiency (kg of seed per kg of fertiliser N) and greenhouse gas (GHG) emissions per tonne of yield. Both NUE and GHGs varied by as much as 30% between elite varieties. In 2007/8, the high yielding varieties had a greater NUE and fewer GHGs per tonne. In 2009/10, there was no relationship between yield and NUE due to the wide variation in N optima which was unrelated to yield. In 2009/10, the variety with the lowest GHG emissions was Grizzly because this variety had the lowest fertiliser N requirement and had a moderate yield. However, it should be recognised that if lower yielding varieties are chosen to minimise GHGs then the area planted to oilseed rape may need to be expanded to maintain production and this may cause indirect GHG emissions as a result of land use change (e.g. converting uncultivated land to arable). The potential effect of land use change on GHGs was estimated in this project and it was shown that when this was accounted for a higher yielding variety (Temple) with a greater N requirement had fewer GHGs than Grizzly. This analysis illustrates that the potential indirect effects of land use change can affect which varieties are judged to be associated with the fewest GHG emissions.

Varieties may affect the risk of nitrate leaching through differences in the size of their N requirement and the amount of N taken off in the seed. In the 2009/10 N response trial the greatest difference in N optima between high yielding varieties was between Temple (245 kg N/ha) and Lioness (307 kg N/ha). After accounting for the differences in N optima, yield and N offtake, positive N balances following harvest were estimated of 100 kg N/ha for Temple and 153 kg N/ha for Lioness, which would be expected to result in a substantial difference in the risk of nitrate leaching during the winter following harvest. It was also estimated that breeding for the low N ideotype described within this project (see section below) would reduce the N balance by 90 kg N/ha compared with a typical crop, which again would substantially reduce the risk of nitrate leaching. Additionally, the low N crops are expected to have a lower N concentration within their crop residues which will reduce the rate of mineralisation and further reduce the risk of nitrate leaching.

2.3.7. Prospects for developing new varieties with low N requirement

The N model developed within the project was used to estimate that if the best varietal traits observed for post-anthesis N uptake, seeds/m², seed N concentration, stem N remobilisation and a 10% increase in fertiliser uptake efficiency could be combined into one variety then the fertiliser requirement for high yields would be reduced by 40%.

The minimum fertiliser requirement compatible with high yields was estimated by calculating the minimum amount of N required in the canopy to intercept and use light as well as to provide essential support functions. Published relationships between the optimum tissue N concentrations for photosynthesis and light intensity were used to estimate the minimum N requirement for a canopy with a green area index of 3.5. The minimum tissue N concentration for essential support functions were estimated based on minimum observed tissue N concentrations at crop maturity of 0.54% N in the stems and 0.87% N in the pod walls. This gave a total N requirement for photosynthesis and support of 153 kg N/ha which compares with a crop N content of 200 kg N/ha for a typical crop yielding 4 t/ha dry matter with a seed N concentration of 3.0% and a N harvest index of 0.60.

Soil N uptake efficiency was based on values observed within this project for the best variety and it was assumed that fertiliser uptake efficiency could be increased from a typical value of 60% to 66%. After taking all these improvements into account the fertiliser N requirement for a high yielding crop grown with a soil N supply (crop N uptake plus soil mineral N measured in February) of 70 kg N/ha was estimated at 92 kg N/ha. This compares with a current N fertiliser recommendation for this soil N index of 220 kg N/ha, thus equating to a reduction in fertiliser N requirement of 58%. It was calculated that reducing the fertiliser N requirement from 220 kg N/ha to 92 kg N/ha will reduce the GHG emissions for a crop yielding 4 t/ha from 1045 kg CO₂e/t to 642 kg CO₂e/t, a reduction of 39%. It was also calculated that if all the photosynthetic N (96 kg/ha) in the minimum N crop was relocated to the seed then the seed N concentration would be 2.4% which is much lower than is typically observed (3.0%).

2.4. Discussion

2.4.1. Conclusions

- Statistically significant interactions were found for seed yield between variety and the supply of N, indicating that different traits are required to maximise yield under Low N supply compared with High N supply.
- Differences between varietal yields under Low and High N supply were generally consistent between seasons indicating that the traits conferring high yield under Low N are heritable.
- Traits for high yield under low N supply identified in field experiments include:
 - Post-flowering N uptake, more seeds/m², high remobilisation of stem N to pods and seed, tall stature, late maturing, high seed moisture, high seed oil, low seed N concentration, high seed glucosinolate concentration.
- Post-flowering N uptake was one of the most important traits and this appeared to be determined by demand from the sink (seeds/m²).
- A mechanistic model of yield determination under N limited conditions was developed which predicted similar key traits for high yield under low N supply as observed in the field experiments.
- Economic optimum N rates varied by over 100 kg N/ha between elite varieties.
- Yield at Low N may indicate low N optima, further testing required.
- Spectral reflectance indices were identified that could be used to predict yield under High or Low N supply from flowering onwards.
- Canopy temperature was useful for predicting yield when used in sunny conditions.
- A genetic map was created for a relevant doubled haploid (DH) population from a cross between elite winter OSR varieties Rocket and Capitol which can be used to associate genetic markers with traits.
- The majority of a set of 75 Tapidor x Victor substitution lines has been genotyped.
- Contrasting yield performance under low and high N supply was identified for specific Rocket x Capitol DH lines and Tapidor x Victor substitution lines which will help identify the genetic regions that control N requirement.
- N efficient varieties require low N optima AND high yield in order to be economically competitive and to minimise GHG emissions.
- GHG emissions varied by 30% between elite varieties as a result of differences in N requirement and yield.
- Breeding varieties with a low N requirement will reduce the risk of nitrate leaching during the winter following oilseed rape harvest.
- It was estimated that the fertiliser N requirement of typical current varieties could be reduced by 58% without reducing yield potential, which would reduce GHG emissions by 39%.

2.4.2. Future R&D resulting from this project

Results from this project demonstrating significant differences in fertiliser N requirement between oilseed rape varieties helped to develop a successful project proposal to Defra 'Project IF01110 - Development of appropriate variety testing methodology for assessing nitrogen requirements of new varieties in trials undertaken for national listing'. The overall objective of this project is to develop the most cost-effective means of identifying the N requirements of wheat and OSR varieties that are candidates for introduction into the UK market.

A Crop Improvement Research Club (CIRC) proposal was submitted in July 2011 entitled 'Identifying traits and genes to lower the fertiliser N requirement of oilseed rape'. The aim of this proposal is to improve understanding of NUE in OSR to underpin the commercial breeding of cultivars for sustainable production in the UK. The project will identify root traits for enhanced N uptake and stem N traits for pod stay green and N utilisation in two DH populations and QTL for these traits and develop resources for future high resolution genetic dissection of the loci using near isogenic lines. This addresses several of the key themes of the BBSRC CIRC call, namely to develop tools for redesigning OSR crops for a step change in NUE. The proposed work will exploit traits and QTLs identified in LK0979 to address improvement of NUE. The work will also make use of the phenotyping data and genetic map developed for the Rocket Capitol DH population in LK0979.

Further R&D is being carried out by individual consortium members to develop the light reflectance crop sensors to enable them to reliably predict yield and help to better select new varieties and target crop inputs.

The core varieties used in this project to identify key traits associated with low N requirement are being further investigated within the oilseed rape genetic improvement network (OREGIN).

Biogemma and the John Innes Centre have indicated that they plan to generate a linkage map of the Rocket x Capitol DH population using about 300 markers. The LK0979 project partners have agreed that they are willing for the map data generated under LK0979 to be added to the Biogemma data to generate a joint higher resolution map.

It is anticipated that with the recent advances in genotyping and sequencing technologies the substitutions in the TVSL population will be located with high precision in the near future which will enable a more thorough evaluation of the data from this project.

2.4.3. Industrial relevance and plans for future commercial exploitation

The success of using light reflectance crop sensors and canopy temperature sensors to predict differences in oilseed rape yield are highly relevant to the industry. Consortium members are carrying out further R&D on these sensors in order to develop them to a point where they can be commercially exploited.

This project demonstrated that oilseed rape varieties can differ significantly for their N fertiliser requirement. This discovery is highly relevant for the industry and has led to a new Defra project IF01110 (described above) to develop a method of identifying varieties with a low N requirement which is closely involved with several plant breeding companies. It is hoped that this project will lead to a method of selecting for low N varieties that can be commercially exploited.

The traits associated with low N requirement that are easy to assess (e.g. maturity date, height, seed N concentration) may be used by plant breeding companies to help identify varieties with lower requirement for N fertiliser.

The project has demonstrated that greenhouse gas (GHG) emissions associated with oilseed rape production can be significantly reduced through varietal choice and future plant breeding to reduce N fertiliser requirement. Reducing GHG emissions is of great importance for the biofuel industry who must meet challenging targets for producing low carbon biofuels.

The understanding developed within this project about varietal differences in fertiliser N requirement will help fertiliser companies to develop guidance for optimising N fertiliser use.

2.4.4. Publications

- Berry, P., Spink, J., Foulkes, J. & White, P.J. 2010. The physiological basis of genotypic differences in nitrogen use efficiency in oilseed rape (*Brassica napus* L.). *Field Crops Research* 119, 365-373.
- Berry, P., Teakle, G., Foulkes, J., White, P.J. & Spink, J. 2008. Breeding for improved nitrogen use efficiency in oilseed rape. 5th ISHS International Symposium on Brassicas and 16th Crucifer Genetics Workshop, Lillehammer, Norway, 8-12 September 2008, 40 (Talk). *Acta Horticulturae* 867, 97-102.
- Berry, P., Foulkes, J., White, P.J., Spink, J. & Teakle, G. 2008. Breeding for improved nitrogen use efficiency in oilseed rape. Association of Applied Biologists, Resource Capture by Crops: Integrated Approaches, Sutton Bonington, 10-12 September 2008 (Talk).
- Berry, P., Teakle, G., Foulkes, J., White, P.J. & Spink, J. 2008. Components of variation in nitrogen use efficiency in *B. napus*. Oilseed Rape Genetic Improvement Network (OREGIN) 6th Stakeholder Forum Meeting on "Assessing low input systems for breeding and trialling - the N and P economy", NIAB, Cambridge, 21 November 2008 (Talk).
- Teakle, G., Durnford, J., Stevenson, S., Foulkes, J., White, P.J., Berry, P. & Pink, D.A.C. 2008. Genetic diversity for nitrogen use efficiency traits in oilseed rape. 5th ISHS International Symposium on Brassicas and 16th Crucifer Genetics Workshop, Lillehammer, Norway, 8-12 September 2008, 86 (Poster).
- Teakle, G., Durnford, J., Stevenson, S., Foulkes, J., White, P.J., Berry, P. & Pink, D.A.C. 2008. Nitrogen use efficiency in *Brassica napus*. UK Brassica Research Community Annual Meeting, Warwick HRI, 21 May 2008.
- Berry, P., Teakle, G., Foulkes, J., White, P.J. & Spink, J. 2007. Reducing the nitrogen requirement of oilseed rape varieties (LK0979). ADAS Open Days, Rosemaund, Boxworth & Mowthorpe, June 2007 (Poster).
- Crop Production Magazine. 28 September 2006. 'Reducing fertiliser for OSR'.
- Farmers Guardian. 16 October 2006. 'Nitrogen use is fuelling biodiesel debate'.
- Enagri. July 2007. 'Breeding oilseed rape for biodiesel'.
- Biofuels International. August 2007. 'Enhancing the environmental benefits of biodiesel production'.
- Fresh Produce Journal. January 2008. 'Biodiesel, nitrogen and climate change mitigation'.
- Crops. March 2008. 'Crops March – N nutrition for OSR'